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The Removal of Pharmaceuticals During Wastewater Treatment: Can it be Predicted Accurately?

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Abstract

The presence of active pharmaceutical ingredients (APIs) in the environment is of growing concern and effluents from wastewater treatment works (WwTWs) are one of the major sources. This research combines the outputs of a multimillion pound UK programme of work to evaluate the fate of APIs in the wastewater treatment process. A combination of analysis of measured data and modelling has been applied to 18 APIs, representing a wide range of medicinal application and physico-chemical characteristics. Some isomers (for atorvastatin) and metabolites (for sertraline, carbamazepine and erythromycin) were also included. High variability was observed between removal rates for individual APIs between WwTW, which after statistical analysis could not be explained by the nominal WwTW process (e.g. activated sludge or trickling filter). Nor was there a clear relationship between API removal and physico-chemical parameters such as pKa, charge or log Kow. A publically available sewage process model, SimpleTreat 4.0 which has been rigorously validated and is now being used for exposure assessment with REACH legislation for organic chemicals and within the Biocidal Products Regulation by the European Medicines Agency for APIs, was used to estimate removal rates with which to compare with measured data. SimpleTreat provided estimates of removal rates within +/- 30% of observed values for the majority of the APIs measured, with the use of readily available WwTW specific parameters such as flow, total suspended solids and BOD data. The data and correlations provided in this study provide support for any future considerations regarding the management of API discharge to the aquatic environment.

Key words: pharmaceuticals; modelling; removal efficiency; wastewater treatment; activated sludge; trickling filter

1. Introduction

The use of active pharmaceutical ingredients (APIs) is increasing throughout the world owing to the widening array of treatments offered, increasing affordability and availability (particularly over the counter sales) combined with a growing population, of which a greater proportion are increasing in age (Jelic et al., 2011). The main source of occurrence of APIs in the environment is considered to be from human use of pharmaceuticals, the majority of which are used, excreted and discharged into the wastewater system (Gardner et al., 2012; Melvin et al., 2016). Owing to the complexity and cost of monitoring micropollutants in environmental matrices and in some cases, the lack of legislation to drive regulation, the availability of fate data can be limited within the public domain. Consequently, there is increasing scrutiny on the levels of APIs entering and being discharged from WwTW (Comber et al., 2018).

Furthermore, the extent to which of APIs are removed during wastewater treatment can be limited. API removal rates are dependent on concentrations entering the works, the API's chemical structure, solubility, charge, potentially toxicity and the existence of viable bacteria with the requisite catabolic/biodegradative capabilities. It should be noted, however, that specific mechanisms of removal are highly complex and in many cases the contribution of individual factors are poorly understood. Previous studies have demonstrated that API removal efficiency can vary between WwTW treatment technologies and even within a given works. Consequently, the quality of WwTW effluent is currently of interest to the pharmaceutical industry seeking better risk assessments, regulators considering legislation and the water industry in terms of the risks associated with their effluents entering the aquatic environment (Gardner et al., 2013).

The range of concentrations found for pharmaceuticals studied in the UK is similar to that observed in continental Europe as well as in the USA (Ashton et al., 2004 and Hope et al., 2012). Most often published data in the literature shows API concentration of less than 100 ng/l in the surface and groundwater, and below 50 ng/l in treated drinking water (WHO, 2011). This is considerably below the human therapeutic dose and any acute toxic limit values for the vast majority of APIs. There is, however, concern regarding potential toxicity and impacts on antimicrobial resistance to the environment when exposed to mixture of APIs and other chemicals and non-chemical stressors (Bound et al., 2006). Many countries have initiated various monitoring programs to investigate the exposure of APIs and to get a better understanding of the pathways and emission sources (Falås, 2012). The

Chemical Investigation Program (CIP) in the UK is a large ongoing monitoring programme for priority chemicals, including emerging contaminants such as APIs in WwTW influent, intermediate processes and effluent as well as their impacts on concentrations in receiving waters (Gardner et al., 2013). The first phase of the CIP (known as CIP1) was an extensive project that ran from 2012-2015 with the primary aim to investigate the fate of trace substances in influent, effluent and within the WwTW process. The result from this extensive investigation has been reported previously (Gardner et al., 2012; Gardner et al., 2013; Jones et al., 2013, Comber et al., 2014 and Comber et al., 2018). With respect to process data, removal of 11 commonly detected APIs at 25 WwTWs (on 26 occasions) were reported for influent, primary, secondary and where present, tertiary treatment effluents (Comber et al., 2018). The £140 million investment in second phase of the CIP (known as CIP2) builds on the outputs from CIP1 by extending the range to include the monitoring of a larger number of analytes, and by including river sampling upstream/downstream of WwTW discharges to measure impact on receiving waters. In total, over 60,000 samples have been taken, resulting in over 3 million determinations. CIP2 includes data for 23 APIs (including some metabolites and isomers) for influent and effluent at 44 WwTW, sampled on 20 occasions (Figure 1; Comber et al., 2018). Furthermore, CIP1 and CIP2 include sanitary parameters (total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, dissolved and total organic carbon (DC, TOC), nitrate and phosphate (Gardner et al., 2013).

Household wastewater quality will vary depending on such things as behaviour and lifestyle, with many sewerage systems also containing stormwater which may also contain APIs (Munro et al., 2019). The sanitary determinands are measured routinely as they are often listed on permits to discharge effluents to receiving waters. The concentrations of these ‘sanitary’ parameters of BOD, COD, TSS, ammonia define the character of the effluent and provide an indication of works performance based on concentrations (lower concentrations suggest higher works efficiency). The presence of APIs is not measured on a routine basis for most WwTWs owing to cost and lack of legislative drivers. Furthermore, modern risk assessments and chemical management are increasingly reliant on models to predict the fate of chemicals through pathways and fate in the environment. Models often provide predictions of treatment efficiency and effluent concentrations which may then be used in tiered risk assessments and environmental regulation. There are a number of software tools available which to various degrees can model the removal of chemicals through the wastewater treatment processes. Over 20 computer programs developed by academia, environmental agencies and commercial sources have been recognised for predicting fate in WwTW (Crechem et al., 2006).

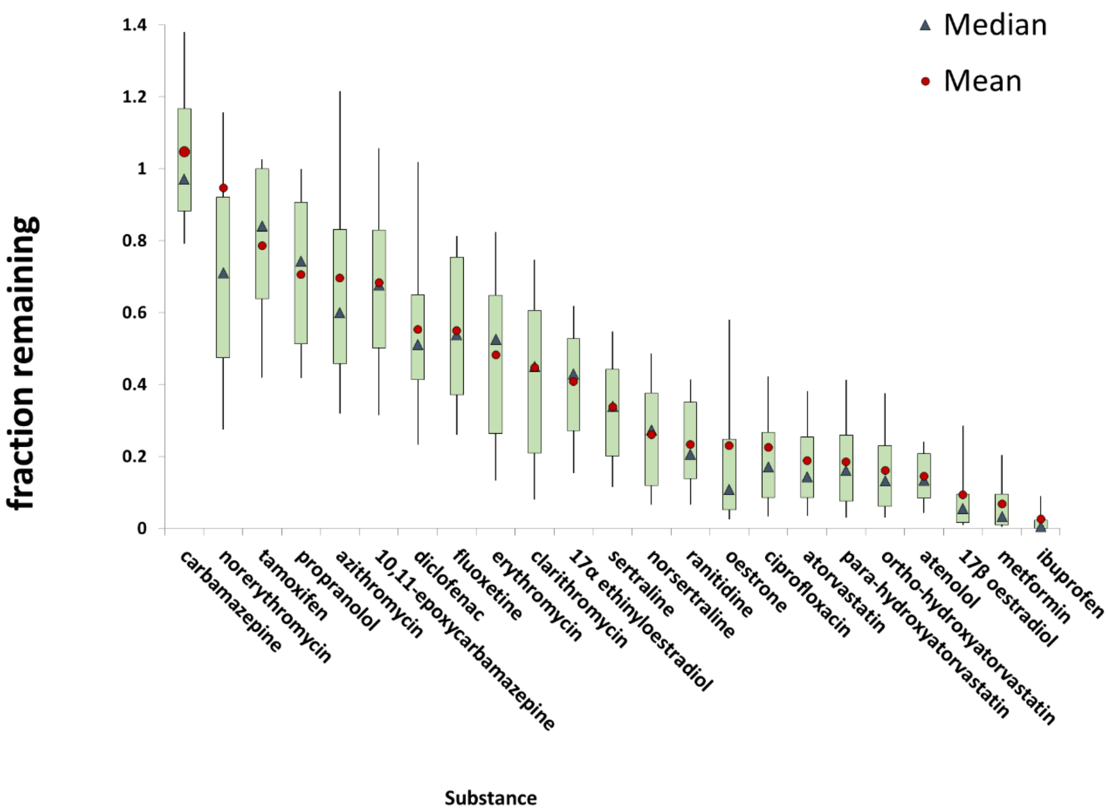

Figure 1: Summary from the CIP 2 program for API median fraction remaining from 44 WwTW sampled on 20 occasions (Comber, 2018). Note the abbreviations used here are used throughout this paper.

SimpleTreat is a fundamental tool used on an official EU level for predicting exposure in the environmental risk assessment. Among others, it is the formally recommended model for the essential assessment for chemical covered in the EU directive of Registration, Evaluation, Authorisation and restriction of Chemicals (REACH), as well as for the market authorisation of new pharmaceuticals regulated by European Medicines Agency (EMA) (Franco et al., 2013; EMA et al., 2006). The tool is straightforward to use and requires the input of a limited number of chemical properties parameters: molecular weight (MW), vapour pressure, water solubility, n-Octanol/Water Partition Coefficient (K_{ow}) as well as the results from biodegradability assessments, as defined by the Organisation for Economic Co-operation and Development (OECD) guidelines (RIVM, 2013). For basic and acidic compounds, the acid dissociation constant, pK_a is also required to take account the state of ionization of polar molecules in the wastewater (Franco et al., 2013). However, it should be noted that many APIs have more than 1 pKa value (although rarely do both occur within expected environmental pH conditions) which cannot be accommodated within the current model and that for ionisable substances such as APIs logD incorporating the ionization potential of the chemical within the partitioning calculations would be potentially an improvement. However, previous studies have suggested that SimpleTreat predicts total removal to an accuracy of ±5% compared with the measured values for the majority of routine wastewater determinands which included non-polar persistent organic pollutants but also ionisable compounds such as triclosan (pKa=8) (Crechem et al., 2006).

Data from CIP therefore offers the opportunity for a detailed examination of the variability of API removal efficiency in light of works type and performance. Specifically, this study utilizes CIP2 program outputs, reporting the presence of 23 APIs (including five metabolites of parent APIs) in influent and effluents, combined with CIP1 data on efficiency of 11 API removal from WwTW secondary process, split into Activated Sludge Plants (ASP) and Trickling Filter works (TF) processes. These data, combined with the use of SimpleTreat modelling, has made possible a critical evaluation of removal efficiency at WwTWs, as well as a comparison of monitoring data with default biodegradation constants provided in the literature and the accuracy of modelling using the accepted risk assessment models. By gaining a better understanding of the key factors controlling the removal of APIs during wastewater treatment combined with an assessment of the effectiveness of modelling will inform future, focused investments as well as more accurate and prioritized targeted risk assessments (Gardner et al., 2013).

2. Materials and methods

2.1 API selection

The selection of chemicals for CIP1 (Gardner et al., 2012) and CIP2 (Comber et al., 2018) are discussed in detail elsewhere. Briefly, APIs were selected based on a risk assessment approach by comparing the estimated environmental concentrations of nearly 150 pharmaceuticals (screened on usage and perceived hazard from a list of approximately thousand candidate substances) with data for their respective effect concentrations on a variety of receptor organisms in the aquatic environment (UKWIR, 2014). For the purposes of CIP2, the list was further refined by selection of substances that were likely to occur in effluents after treatment and that were considered to have the greatest potential as candidates for inclusion on the WFD priority substance list (EU, 2011). This resulted in the list of substances (n=13) tabulated in Table A1 of the Electronic Supplementary Information (ESI).

2.2 Sampling strategy

A set number of WwTW were selected for the CIP1 and 2 programs with the justification for which are described elsewhere (Comber et al., 2018), being based on a combination of low dilution in the receiving water, representative types of works (roughly evenly split between ASP and TF), geographic location (covering England, Scotland and Wales), and size (serving populations between 2,000 and 1.6 million). Owing to the varying hydraulic retention times (HRT) for individual works, which are often not accurately known and can be measured in days (Ejhed et al., 2016) meant it was not practical to try and match collection of influent and effluent related to the HRT of the selected WwTW. However, given the mixing that occurs within a given WwTW, combined with sludge returns, inputs from storm tanks and combined sewers it was decided that sample replication based on numerous sampling occasions would derive statistically robust conclusions regarding WwTW performance.

Data used for this research were (Table A2 in the ESI):

- **CIP1 program**: 25 WwTW data for influent, after primary settlement and final effluent after secondary and if available tertiary process for 11 APIs. Two samples of each process (spaced more than 4h apart to provide a degree of replication) were taken on between 10 and 15 occasions between 2011 and 2013. In this part of the programme two samples.
- **CIP2 program**: Single samples for 18 APIs and 5 metabolites were spot sampled on 20 occasions at 44 WwTWs in the influent and effluent (not intermediate process stages, unlike CIP1) over a two-year period between 2015 and 2017.

A summary of the CIP sampling strategies is provided in Table A3. Grab samples at various time intervals were used for the collection of aqueous samples. To assess variability within the day, in the CIP1 program, at least one duplicate sample was taking during the same day with a minimum of four-hour period between the sampling. Composite samples were not considered owing to concerns regarding sample stability. A minimum of 15% of the samples were taken at non-working hours (evenings and weekends). The sampling schedule was conducted according to stratified random strategy, indicating that the sampling events are spaced approximately evenly during the year at monthly intervals, but are randomly placed at each interval in the month.

2.2 Laboratory analysis

Samples were collected in stainless steel samplers, stored in glass container and transported at 4° C to the analysis laboratories. The samples were stored a maximum of 5 days prior to analysis. The samples for measuring the endocrine disrupting chemicals were preserved by adding 30% hydrochloric acid and copper nitrate (Gardner et al., 2012). The quality assurance/quality control procedures were conducted for experiment preparation, sample collection, sample pre-treatment and analysis for both laboratory tests and field sampling. All the samples were analysed by any of four approved laboratories with ISO17025 accreditation and showed to be able to achieve the analytical performance and quality assurance laid down in the specification (see A1 of ESI). The pharmaceuticals were analysed by LC-MS or GS-MS. The analytical error of all the pharmaceutical measured were considered to be ±50%

(25% random error and 25% systematic error) or the Limit of Detection (LOD) if this value was larger (Table A4). In accordance to EU regulations, if analysed concentrations were below LOD then the value for LOD was halved to generate a result (EC, 2009). There were no major inter-laboratory error and inter-regional variation, which would otherwise indicate if there was a bias in the procedure of sample handling and analysis method. Further details of the proficiency testing can be found in the supporting information (A1).

2.3 Data handling and analysis

The data handling and the statistical analysis were conducted with either Microsoft Excel (2016) or IBM SPSS Statistics software (version 20). This study also made use of the tool SimpleTreat (version 4.0) for modeling fate in WwTW, application developed by the National Institute for Public Health and the Environment (RIVM). EPI Suite (version 4.11) was used for retrieving some of the non-published physico-chemical data, available from the US EPA (US EPA, 2016).

In the data handling, the replicates were averaged, and this value was then used for further statistical calculations. Mean, maximum, minimum and percentiles were calculated from the daily average. Fraction remain was calculated from the influent concentration as a fraction of the various stages of the process. The removal was calculated as percentage from the concentration based on the effluent concentration subtracted from the influent then divided by the influent, expressed as a percentage.

2.4 SimpleTreat 4.0 (RIVM) emission model

The model SimpleTreat 4.0 (RIVM, 2013) was used for estimating the percentage removal in the WwTW for a number of the APIs in the CIP program. SimpleTreat is an established, readily available free to download model often used within regulatory risk assessment frameworks to estimate predicted environmental concentrations for ASP only (not TF WwTW). Input parameters include noting if the chemical is potentially ionisable. Given APIs are often charged, the model accommodates by calculating the proportion of the APIs that is neutral at pH 7.0 and this determines the equation used to calculate the default organic carbon:water partition coefficient (Koc). Molecular weight, K_{ow}, vapour pressure and solubility are other required input variables to the model. Henry's Law Coefficient (H), Organic carbon partition coefficient (Koc), Organic carbon partition coefficient for raw and settled sewage as well as for activated sludge (Kp) can be added as an adjustable input or the model creates a default value.

3. Results and discussion

3.1 Comparison of API data between CIP1 and CIP2

Previous data analysis has shown that the CIP data for APIs in WwTW effluents corresponded well with those reported elsewhere for UK effluents (Comber et al., 2018). To investigate the quality of the data, and to examine if there had been any systematic shifts in API effluent concentrations between the CIP1 the median fraction remaining from both the CIP1 and CIP2 were compared for APIs that were studied in both programs (E1, E2, EE2, IBPF, DCF, FLXT, PRPL and ERMY). Taking account of the significant variability of removal efficiencies for individual APIs, good agreement was obtained between the fraction remaining in effluent of those APIs common to both CIP1 and CIP2 (Figure 2). These results provided confidence in the analytical data obtained between the two separate programmes (using different analytical laboratories in some cases) and that there were no gross changes or variations between the WwTWs selected for sampling or impacts on removal rates associated with the sampling periods (e.g. seasonality) or methodologies used.

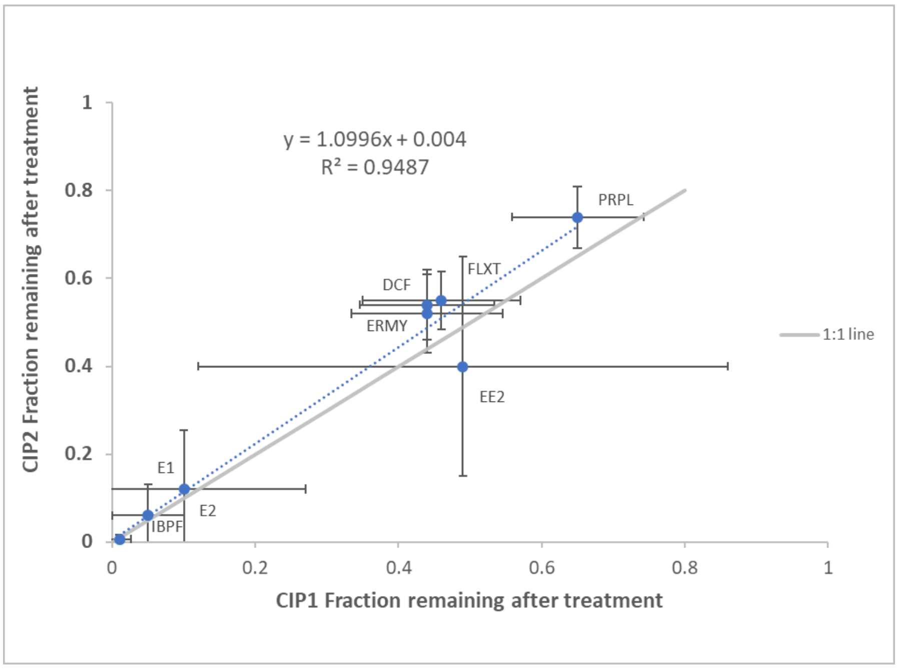

Figure 2: Median fraction remaining after treatment comparing CIP1 (25 WwTW) and CIP2 (44 WwTW) programs. Solid line is the 1:1 line and the dotted line is fitted linear trend line and error bars are 95% confidence intervals. P value = 4.3×10^{-5}.

3.2 Physico-chemical characteristics potentially impacting the API removal in WwTW

APIs can be characterised broadly in terms of their charge and their ability to accept or donate protons; with carboxylic acid APIs acting as acids and amine groups acting as bases under environmentally relevant pH conditions. The degree of dissociation (reported as pKa) is crucial when the ambient pH of the WwTW effluent is close to the value of the pKa of the API. In some cases, where there are carboxylic acid and amine groups present on the same compound, depending on the ambient pH, the molecule may be rendered charge neutral depending on the size of the molecule and spacing between ionisable sites. The pH of sewage effluent is circumneutral and so for assumption of charge and calculation of LogD, a pH of 7 was assumed (Gardner et al.,2012)

This is a particularly important physico-chemical characteristic as the charge on the molecule will in some degree impact on its affinity for particulate matter, complexation/association with organic matter and other counter-ions and affect solubility and partitioning and hence bioavailability to microoganisms (Greenhagen et al., 2014; Tappin et al., 2016). These are all crucial parameters in determining the removal rate during wastewater treatment. As a general rule, biological uptake is mostly associated with neutral molecules, particularly if they are also hydrophobic (Haitzer et al., 1999). Positively charged compounds will show a tendency to sorb strongly to clay minerals and solids which have a predominantly negative charge. Negatively charged compounds therefore generally have lower affinity for sorption and uptake, although for complex molecules with multi-protic sites this is somewhat of an over simplification (Bendz et al., 2005; Katsoyiannis et al., 2007).

There was no clear relationship between removal rates and groups of acid, basic and neutral APIs (Figure 3). Poor DCF removal may be a result of the combination of chemical structure, specifically the presence of halogen functional groups (Verlicchi, 2012) and its hydrophilic nature (log K_{ow}1.5) reducing bioavailability and increasing persistence. As observed previously (Tappin et al., 2016) the data show that it is not possible to accurately predict removal of the selected APIs during wastewater treatment using charge, Log Kow, solubility (LogS), pKa,or LogD which is Kow corrected for the charge on any given molecule for a specific pH (7.0 assumed in this case). The only conclusion which may be drawn is that the majority of the basic APIs show poorer removal, possibly owing to reduced bioavailability of the positively charge molecule (Yamamoto et al., 2009).

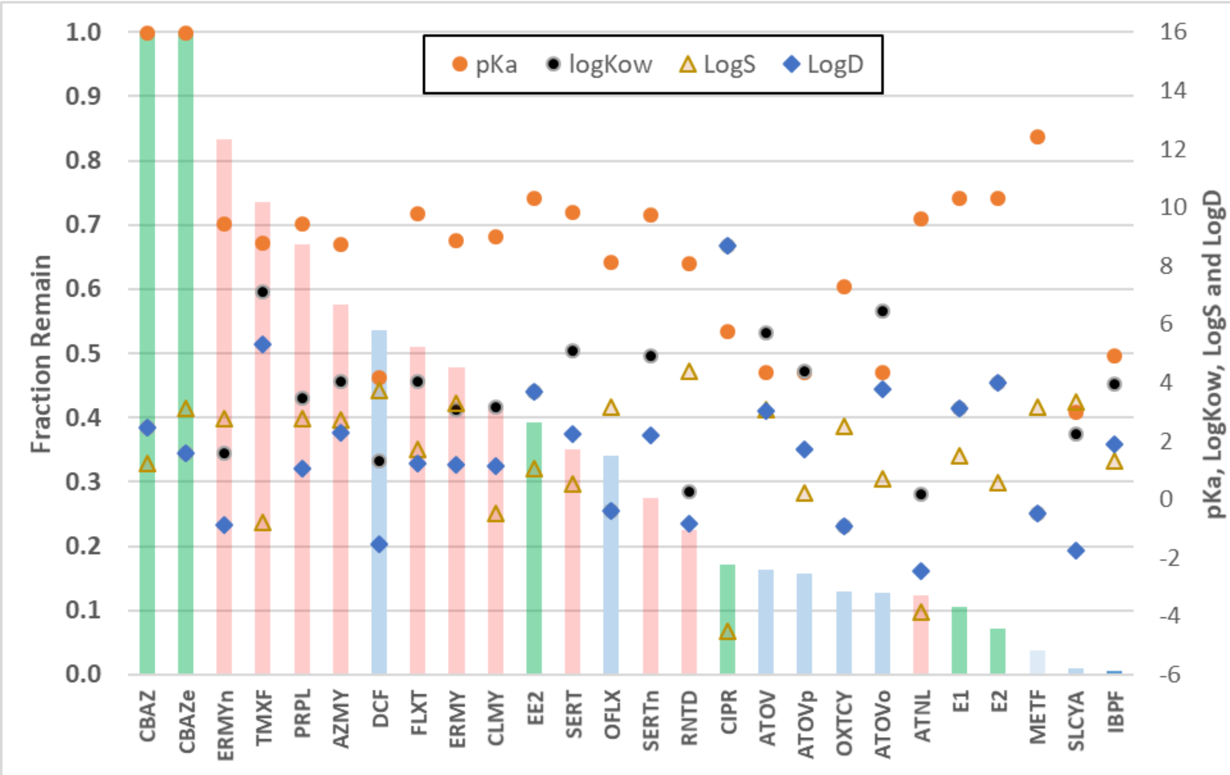

Figure 3: The order of total fraction remaining (median) for APIs as function of pKa, LogS, LogD and logKow for the CIP2 and CIP1 APIs not covered by CIP2 (blue colour for acidic compounds; red for basic; green for neutral or zwitterions)

3.3 Variation in efficiency of API removal by different works technology (ASP vs TF)

Major investments have been made across the UK to upgrade WwTWs from TF to ASP as they are generally more efficient and reliable in removing BOD and suspended solids, as required by permits to discharge to receiving waters (Water UK, 2018). The CIP API data were therefore examined to determine if there were any significant differences in treatment efficiency between TF and ASP (Figure 4, Table A5). The CIP1 data contained 9 TF and 13 ASP WwTWs and the CIP2 data compared 15 TF and 18 ASP WwTWs APIs percentage removal. For the CIP1 data (Table A5) the secondary process was separated out (i.e. not total percentage removal) to provide a more accurate comparison with the CIP2 data.

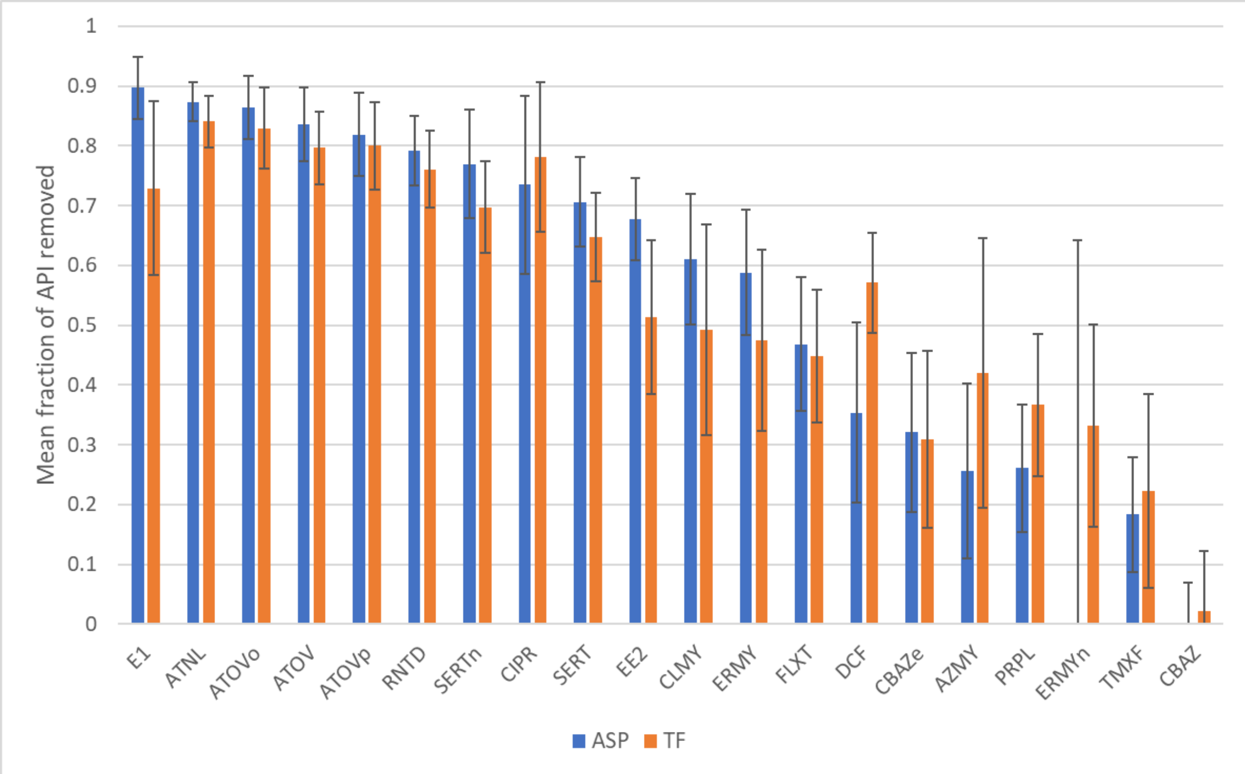

Figure 4: CIP2 mean fractional removal rates for ASP and TF WwTW with 95%ile error bars.

Data from both CIP1 and CIP2 data, indicate that although in many cases the mean performance for API removal at ASP works is better than that for TF, which has been reported elsewhere for a different set of chemicals (Falås, 2012) however, for none of the 23 compounds measured was the difference statistically significant. What is also noteworthy is the fact that for APIs where removal may be considered good (e.g. greater than 70%) then variance between works (ASP and TF) are generally lower than where removal rates are poorer. These data therefore indicate that the type of technology is less critical for the overall removal efficiency of APIs than WwTW specific processes and characteristics such as hydraulic retention times, sludge retention times, sludge return management and biodegradability of the API itself. Another potentially complicating factor is API conjugation. Metabolic transformations include glucuronidation, sulphation, acetylation of the parent API to increase solubility and aid excretion. Conjugated metabolites can undergo retransformation back to the parent form following cleavage of the conjugated moiety which has been hypothesised to occur within WwTW for estrogens, carbamazepine and diclofenac which are included in the CIP suite of determinands. Although the potential significance of deconjugation during wastewater treatment has been acknowledged, detailed empirical evidence is still scarce, being limited to estrogens, because of analytical challenges (Polesel et al., 2016; Brown and Wong, 2018). As a consequence discussion relating to absolute removal rates have to be viewed in this light, although comparisons between different processes is more of a relative comparison.

3.4 The relationship between sanitary determinands and pharmaceutical removal

The benefit of gathering concentration data regarding the sanitary determinands (AMON, BOD, COD and TSS) in combination with that for APIs, allows the ability to seek correlations between metrics which indicate the overall performance of a WwTW with respect to API removal. If such relationships can be established, then there are multiple benefits:

- Majority of the WwTW routinely measure the sanitary determinant so this data is already available. The ability to predict a WwTW's potential API removal efficiency based on a cheap and readily available sanitary determinants analysis data, without any issues possibly associated with time delays with the analysis method and sampling strategy for APIs (Roberts, 2006).
- By extension, the capability of being able to apply the outputs into available models (like for example SimpleTreat) predicting API removal based on input variables associated with TSS, AMON, BOD etc.
- Ultimately, allow the potential for optimising WwTW operations (through for example, hydraulic retention time, increased biological treatment, use of coagulants etc.) to achieve the desired API removal efficiency without additional expenditure on tertiary treatment.

Many UK WwTW receive a combination of both crude sewage from domestic and industrial sources and surface water runoff. Runoff contributes flow but is unlikely to contain APIs or significant BOD. Industrial discharges are often rich in BOD but their flow in most cases is insignificant compared with that from domestic sources. Flows and loads of down the drain chemicals such as APIs to WwTW vary within and between days and seasons; furthermore, the proportion of loads from industrial and domestic flows may also vary. Consequently, WwTW capacities are generally described as population equivalents (PE) which is the normalised unit per capita loading, representing the ratio of the sum of the pollution load produced during 24 hours by industrial facilities and services to the individual pollution load in household sewage produced by one person in the same time. Given that population and consented flow data were available for all WwTW (Table A6), an analysis of normalised data was carried out by multiplying the individual WwTW flow (measured where available, consented otherwise) and then dividing by the PE, thereby taking account of individual WwTW demographics.

Any observed correlation between API removal and sanitary determinands is likely to reflect a combination of works efficiency and API physico-chemical characteristics (Table A7). For example, a WwTW with high TSS removal suggests efficient settlement and sludge separation and so APIs with a high tendency to sorb to solids (i.e. high log Koc); alternatively, a high BOD and API removal correlation suggests the API is susceptible to biodegradation or co-metabolism. Correlations do not necessarily mean a cause and effect relationship, so there may be other factors influencing the correlation. Furthermore, this would suggest that the process parameters (PE and flow) probably do not account as the only factors for the observed variation in API removal between various plants. PE and measured/consented flow is an indication of the burden of the plant due to for example the population size and the industries present in the area; but these are static values and do not take into account the variability within the year. However, these variations can be seen when looking at the correlation between the measured sanitary determinands and the APIs removal in the WwTW. It was found that with or without normalisation of the data the correlation between sanitary determinants and API analysis concentration was not sufficiently good to allow useably accurate predictions of API concentrations from sanitary determinand surrogate data (Table A6). There were also no differences seen when separating out data from TF and ASP technology processes.

To move beyond simple correlations a Principle Component Analysis (PCA) was performed on the influent and effluent CIP2 data, where the proximity of determinands on the charts would suggest a degree of relationship/co-variance. (Figure 5). The data presented, however, largely supports that generated from the correlation analysis (Table A7). For the influents it can be seen that the sanitary determinands (BOD, COD, TSS, TP and DOC/TOC) are grouped together showing the expected strong signal from domestic wastewater which would be likely to contain similar ratios owing to a common source. The APIs do not relate to the sanitary determinands, most likely owing to their inputs relating to prescription and/or seasonal use. For the effluents a slightly different pattern is observed. The sanitary determinands are more separated, likely to be a result of varying treatment (i.e. a potential bias for TSS removal during primary treatment and BOD by secondary treatment). The APIs reflect this with certain APIs (e.g. E1, E2, IBPF, ATOVp, METF) more associated with their biodegradability and so align with BOD. In other words, high performing works reducing BOD to very low levels, are likely to also reduce the concentrations of more easily degradable APIs. Overall, the lack of clear and distinct groupings reflects the complexity of removal mechanisms related to this class of compounds as well as the potential influence of API de-conjugation during the sewage treatment process (Brown and Wong, 2018). Overall, it may be concluded that although there appears loose associations for certain physico-chemical parameters for certain classes of APIs, the biodegradation and partitioning processes with sewage treatment are highly complex and likely to include other interactions such as electrostatic, complexation and cation-bridging mechanisms which would be likely to interact with APIs and thus influence their sorption behaviour and bioavailability (Toll, 2001). However, given APIs often exhibit low logKow (<4.0) and high solubility, their interaction with the particulate phase during primary treatment would be expected to be less significant than potential biodegradation loss mechanisms during secondary treatment (Table A7).

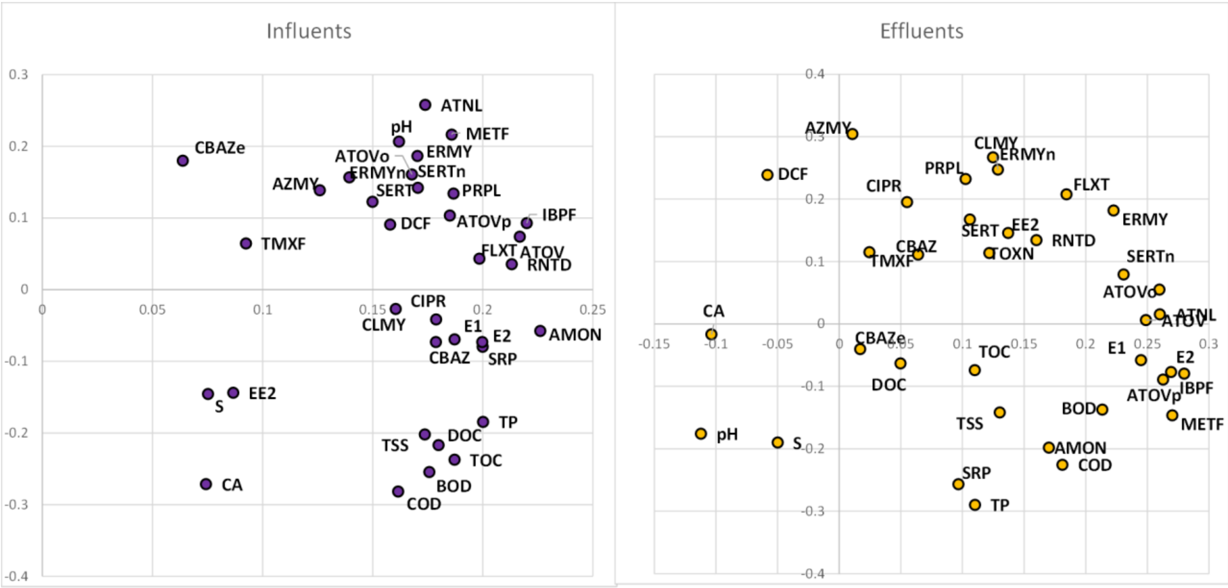

Figure 5: Principal component analysis (axes unlabelled as simply pca1 and pca2) of the influents and effluents for CIP2. CA=calcium; S=sulphur; TP=total phosphorus; SRP=soluble reactive phosphorus; TOXN=total oxidisable nitrogen.

3.6 SimpleTreat 4.0 (RIVM) emission model

The observed variability in estimating API effluent concentrations from sanitary determinands leads onto the question of whether established models used within the risk assessment process can provide a better outcome. The freely available model SimpleTreat 4.0 was used for estimating the percentage removal in the WwTW for a number of the APIs in the CIP program and predictions compared with observed data from the CIP datasets. The ASP process can be left default or site-specific flow, sewage solids and BOD can be inputted along with loading rate and pH. Surface aeration (default) or bubble aeration can be selected as mode of operation. For the purposes of this exercise, given that flows, BOD and TSS were available for individual WwTW they were input into the model to generate a degree of WwTW-specific outputs. The key and most sensitive variable however, is the biodegradation rate employed for the secondary treatment process (hr^{-1}). Data for biodegradation, in particular official OECD testing data, is not readily available in literature for APIs. A series of defaults are available based on standard OECD tests which indicate if a compound is readily biodegradable (1 hr^{-1}), readily biodegradable, failing the 10-day window (0.3 hr^{-1}) and inherently biodegradable fulfilling specific criteria (0.1 hr^{-1}). Inherently biodegradable, not fulfilling specific criteria or not biodegradable are assumed to be persistent (0 hr^{-1}). However, for APIs a OECD 301 biodegradability assessment is not mandated if OECD 308 data are generated, provided the pharmaceutical passes the Phase 1 of the tiered assessment approach, in other words, it has a $PEC_{surfacewater}$ <10 ng/l and log Kow >4.5 and as well as certain mode of action (EMEA, 2006). Consequently, not all the APIs in the CIP program could be estimated in the models (Table A9). When API removal data from both CIP1 and 2 was available, an average value was used for comparison with SimpleTreat predictions (Figure 6).

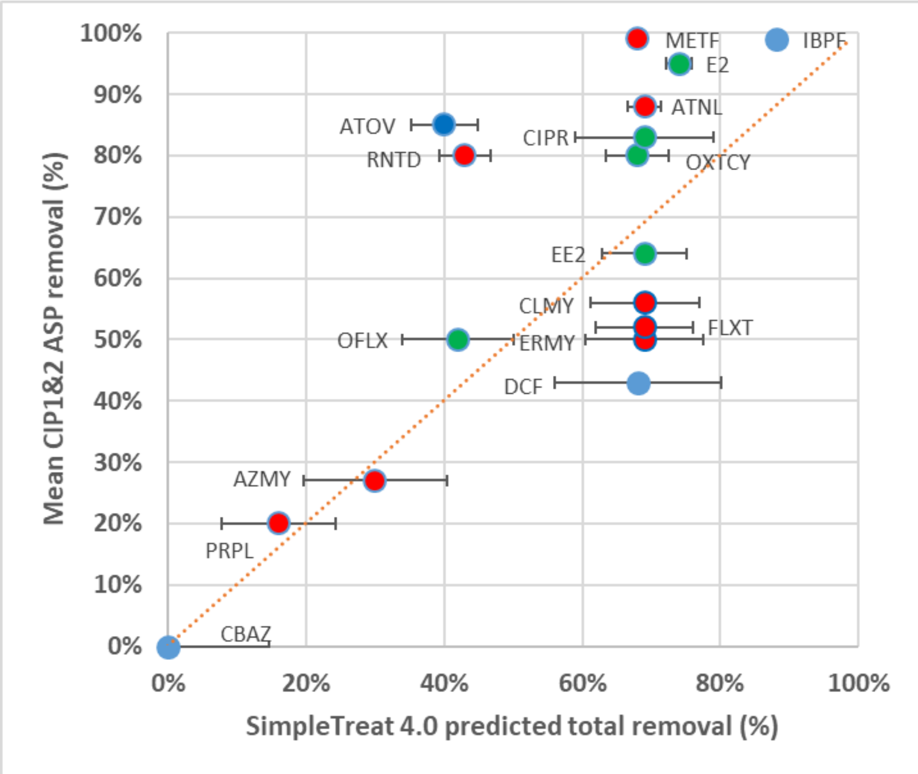

Figure 6: SimpleTreat 4.0 predicted removal versus measured data with 95% confidence intervals for the CIP1 and CIP2 data (red dotted line=1:1; blue colour for acidic compounds; red for basic; green for neutral or zwitterions)

Overall good agreement was obtained between SimpleTreat and the CIP measured data, with 13 of the APIs predicted to be within 30% of the CIP measured value, with no obvious systematic bias. This is in agreement with previously reported assessments (Crechem, 2006). In broad terms, there tended to be better agreement for neutral/zwitterionic APIs than for the charged compounds (at ambient wastewater pH). In general, it was found that SimpleTreat tended to under estimate the percentage removal for 10 of the APIs, particularly for those more readily degraded, which being conservative (i.e. there is greater removal in reality than predicted, so less API is being discharged than predicted) meets the precautionary principle for risk management (UN, 1992). However, this places potential costs on society that are not warranted, so it needs to be applied as a screen for further validation.

Furthermore it was possible to reverse engineer biodegradation rate constants for API removal during secondary treatment using the SimpleTreat 4.0 model. For CIP1 ASP WwTW data were collected for influent, as well as after both primary and secondary treatment, unlike the CIP2 WwTW where only influent and effluent concentrations were measured. Consequently the CIP1 dataset allowed the efficiency of secondary treatment alone to be calculated as a percentage of API removal. For each of the CIP1 WwTW where API concentrations were greater than the limit of detection, API characteristics, flow, BOD and TSS were input into SimpleTreat and the secondary treatment biodegradation rate adjusted until the predicted percent removal of the API matched that observed at the WwTW. This generated a series of rate constants for biodegradation for 9 APIs for between 7 and 13 WwTW secondary processes. The mean, median and range of these derived rate constants could then be compared with default constants generated from OECD laboratory tests that are applied in models as risk assessment to critically assess their efficacy under real-life conditions (Table 1).

Table 1: Reverse engineered default rate constant generated by SimpleTreat 4.0 using CIP1 secondary ASP removal data.

API	Default Rate constant (hr^{-1})	SimpleTreat 4.0 fitted secondary treatment rate constant for CIP 1 ASP (hr^{-1})					
		mean	**sd**	**median**	**n**	**min**	**max**
DCF	0.3	0.02	0.02	0.003	13	0	0.1
ERMY	0.3	0.22	0.42	0.038	9	0	1.3
FLXT	0.3	1.99	2.5	0.325	8	0.002	5
EE2	0.3	1.77	2.18	0.39	11	0	5
IBPF	1	0.91	0.54	1.1	9	0.15	1.5
OXTCY	0.3	0.67	1.34	0.22	13	0	5
OFLX	0.1	0.84	1.69	0.062	9	0.032	5
PRPL	0.002	1.19	2.01	0.038	7	0.019	5
E2	0.3	2.81	2.15	2.2	11	0.3	5

By using a combination of the SimpleTreat model and observed CIP1 secondary removal data, it was possible to fit a biodegradation rate for secondary treatment and compare it with default OECD derived values (Table 1). Firstly, given the variability in the datasets, fitted first order degradation rates varied considerably, with maximum and minimum varying by 2 orders of magnitude in some of cases, although all of the APIs tested, apart from DCF, default degradation rate lay between the observed minimum and maximum value. As already notes DCF, the steroid estrogens and CBAZ may be susceptible to undergo de-conjugation during the treatment process and so observed 'removal rates' may not reflect modelled assumptions or ready test biodegradation data; although the latter would be subject to similar possible microbiological interactions (Brown and Wong, 2018). The median CIP1 fitted degradation rate was within a factor of 2 of the default for OFLX, OXTCY, FLXT, IBPF and EE2; within an order of magnitude for ERMY and E2, but the default rate constant was considerably higher for the anionic DCF and lower for the cationic PRPL. In regulatory risk assessments it is often assumed that there is zero WwTW removal and in most cases there are no risks and hence there is little need to refine; hence few WwTW data are currently generated. However, from a conservative risk assessment point of view, a default degradation rate being lower than observed is desirable, as it will lead to an over estimate/worst case for effluent concentration and hence PEC. This was the case for four of the APIs, but given that another three were within a few % of the fitted values, as well as E2 and PRPL, where PECs could be generated significantly lower than likely observed concentrations, owing to the over optimistic degradation rates being applied. However, taking account to that the WwTW conditions of BOD, TSS, partitioning to sludge etc, overall removal rates for PRPL are close between observed and predicted, although DCF SimpleTreat removal estimates are significantly higher than observed, owing to the much higher degradation rate applied.

Overall, the SimpleTreat estimates of API removal are encouraging and the application of easily available WwTW metrics (flow, TSS, BOD) allows accurate predictions to be used which would allow for tentative risk assessments to be undertaken where measured data are not available.

Finally, it is important to consider the wider impacts of these finding, particularly relating to the risk assessments required for chemicals likely to enter the environment. Provide sufficient data is available then a similar approach should be able to be applied to other substances of concern that occur in wastewater including illicit drugs, pesticides and other classes of APIs such as antiretrovirals (Munro et al., 2019). Furthermore, reverse engineering biodegradation half-lives using monitoring data is quite an expensive way to achieve this and can only be done reliably once an API is in patient use and after WwTWs have adapted to potentially biodegrade the compound. APIs are 'down the drain' chemicals and current regulations from the EMA require the determination of LogKoc and LogKow as well as the OECD 301 (ready biodegradability) and 308 (aerobic and anaerobic transformation) tests. Using SimpleTreat to reverse fit secondary treatment biodegradation rates showed that a wide variation in rate constants are generated, reflecting the observed data, with median values which can differ considerably from values generated from OECD ready biodegradation tests. The likely reason for these differences are the artificial conditions used within such tests, in particular, fixed temperatures, elevated API concentrations, low biomass concentrations and variable inoculums (Martin et al.,2018). There is no requirement to conduct a 314B (activated sludge die-away) or 303 (aerobic sewage simulation) tests within the required ERA for EMA. Given the variation in removal observed at WwTW and the need to get a realistic PEC for surface waters, so that those APIs of greatest risk can be prioritised, the EMA guidelines may need to be amended to reflect this. This might include giving greater consideration to WwTW removal in Phase II Tier A and/or B. The draft revision out for consultation (EMA, 2018) allows the OECD 301 test to be waived if the OECD314B test has been completed, which is a positive move and the results presented here do support the need for greater consideration of WwTW within the ERA process. The application of this approach might also help the water industry to prioritise on those drugs with low removal much earlier.

4 Conclusions

The removal of APIs observed between and within the individual WwTW is shown by CIP monitoring to be highly variable and of greater significance than any variance between overall type of treatment (e.g. ASP versus TF). There was no usable correlation found between concentrations of sanitary determinands such as AMON, BOD, COD and TSS and observed those of APIs. The only conclusion that could be drawn was that high performing WwTWs (with high levels of sanitary determinand removal) lead to the strong likelihood that APIs too, will be more effectively removed. Relatively accurate estimates of removal were achievable using the latest version of the SimpleTreat model for ASP WwTWs, which accounts for the charge present, a significant (but not only) controlling factor in the fate of APIs during wastewater treatment. SimpleTreat was capable of predicting API removal with an uncertainty of +/- 30% for the majority of the APIs tested, based on readily available WwTW specific parameters such as flow, total suspended solids and BOD. This has been achieved without any account of processes such as de-conjugation which is poorly understood at the present time.

Overall, it may be concluded that SimpleTreat using some easily obtainable WwTW parameters such as TSS and BOD concentrations, offers a relatively refined modelling option for API risk assessment purposes, provided there is confidence in the degradation rate constants used. The data and modelling presented here supports the move towards greater consideration of WwTW within the ERA process for APIs.

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